THE RELATION BETWEEN MYCORRHIZAE AND THE DEVELOPMENT AND NUTRIENT ABSORPTION OF PINE SEEDLINGS IN A PRAIRIE NURSERY[1]

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The importance of mycorrhizae in the normal growth and development of forest trees has been, and still is a highly controversial question. Sufficient evidence has been accumulated during the past few years to leave little doubt of their importance in the normal growth and development of coniferous seedlings. Indeed, under certain conditions they appear essential. Such a case is described in the following article.

FOR a long time ectotrophic mycorrhizae have been known to occur on the root systems of many trees, especially the conifers, but few people realize their almost universal occurrence. Various theories have been advanced concerning the presence of mycorrhizae, how they function, and whether or not the fungi concerned in their formation are parasitic or symbiotic. Among the authors of papers on mycorrhizae there has been considerable disagreement and none of the theories advanced has explained completely many of the known facts.

Recently Hatch (3) has completed a comprehensive work dealing with mycotrophy in the genus *Pinus*. This work reemphasizes the significance of mycorrhizae in forestry and particularly in afforestation. Hatch's conception of the role of mycorrhizae, based upon a critical review of the published data of others, and upon his own experiments, is that mycorrhizae occur normally only in soils where there is a deficiency in one or more of a number of mineral nutrients. This theory was originally put forth by Stahl (9) in a slightly different form and is a considerable enlargement on the organic nitrogen theory[2] which in the past has had wide adherence (5).

According to Hatch's conception tree roots in soils deficient in one or more nutrient elements are attacked by mycorrhizae-forming fungi. As a result of the mycorrhizal formation, trees growing in these poor soils are able to obtain a more adequate supply of mineral nutrients for the following reasons: the greater surface area of the infected short (mycorrhizal) roots; the greater abundance of the absorbing root ends due to the profuse branching of the mycorrhizal structure; the delay in suberization of the cortex and endodermis of the infected short roots; and the great extension of the fungal mycelium through the soil and the resultant increased surface for mineral absorption. Hatch's experiments, as well as those of Mitchell et al. (6), furnish proof of the role of mycorrhizae in aiding trees to absorb greater quantities of minerals in which the soil is particularly deficient, especially phosphorus, nitrogen, potassium, and calcium. These experiments indicate the obsolescence of the idea that tree mycorrhizae are usually non-symbiotic structures, and also of the conception that they are concerned only with the absorption of nitrogen in raw humus soils possessing few nitrates.

The present paper presents data which support the main thesis of the mineral nutrition theory of mycorrhizae. It con-

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[2]Melin's later work apparently indicated the inadequacy of the organic nitrogen theory in explaining all mycorrhizal data.

trasts the results of growth and mineral absorption in mycorrhizal and non-mycorrhizal seedlings of Virginia pine (*Pinus virginiana* Miller) grown in a prairie nursery.

Experimental

In the spring of 1937 a new tree nursery was established at Ames, Iowa. This nursery was located on O'Neil sandy loam; a light black, high terrace soil ranging in pH from 5.8 to 6.5 and underlaid with gravel at a depth of 2 to 3 feet. The nursery site had been farmed for many years, but probably originally supported a sparse growth of oaks, shrubs, and native grasses.

In the coniferous section of the nursery a number of species of pines were planted. These included northern white pine, ponderosa pine, Austrian pine, red or Norway pine, Japanese red pine, and Viriginia pine. The pine beds were all planted in the interval from May 15 to May 30. Following planting, all of the beds, excepting those of red and Austrian pine, were mulched with pine needles brought in from a vigorous growing plantation of red and white pine in the near vicinity.

lack of Ca!

Germination was good in all species except the Austrian pine, and all of the seedlings developed normally until about the first of August when the first fascicled needles were forming. At this time it was noted that in the beds mulched with pine needles there were certain spots where the seedlings appeared more vigorous than in the rest of the beds. As the growing season progressed these spots of seedlings remained green and vigorous while the remainder of the seedlings developed a stunted appearance and turned brown to reddish purple in color. This spotting occurred in all the beds mulched with pine needles although in the white pine bed the spots were not so pronounced and all of the seedlings appeared slightly yellowish green.

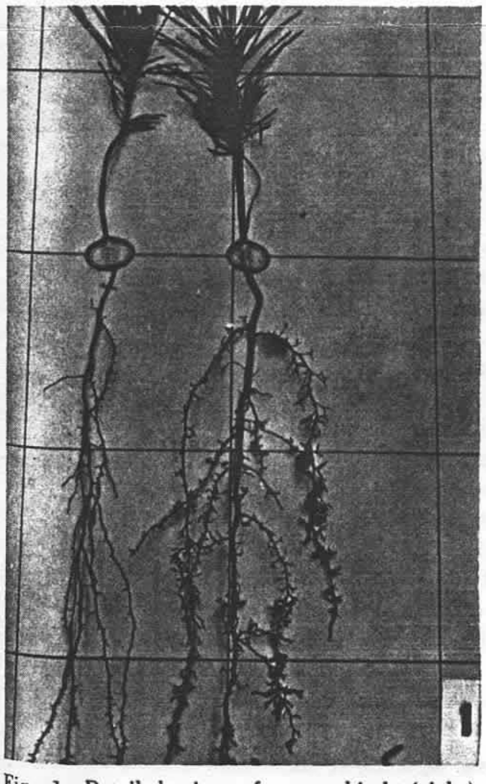

Fig. 1—Detailed view of mycorrhizal (right) and non-mycorrhizal (left) seedlings of *Pinus virginiana.*

Upon searching for the cause of the spotted appearance of the beds, it was found uniformly that the vigorous seedlings possessed an abundance of ectotrophic mycorrhizae, while the stunted plants possessed few or none. This relationship was true of all the species mulched with needles although mycorrhizae were particularly abundant and their influence more pronounced in the case of the Japanese red and Virginia pines.

Because the outward manifestations of the mycorrhizal habit were more evident in the two species mentioned, one of them (the Virginia pine) was used in obtaining quantitative data. To obtain these data, a block of soil containing over 200 seedlings was removed from each of a mycorrhizal and non-mycorrhizal spot in the seed bed. The two blocks were within ten

feet of each other in the seed bed, and the soil pH between the blocks varied from 6.0 to 6.2. The soil was carefully washed from the seedlings and the damaged seedlings at the edges of the blocks thrown away. From the uninjured seedlings a sample of 20 was chosen at random from each lot. The remainder of the seedlings in each lot was divided into four samples, the number of seedlings in each sample varying from 25 to 50.

The twenty mycorrhizal and twenty non-mycorrhizal seedlings were examined to determine the nature of the mycorrhizae and counts were made of the number of mycorrhizal branches or tips and of the non-mycorrhizal short roots. The root classification of Hatch and Doak (4) was used. The same seedlings were used in measuring height growth. The following measurements of height growth were taken: from root collar to top of cotyledonary growth, from the top of cotyledonary growth to the bud, and total height. The growth from the cotyledons to the bud was believed to be most useful in evaluating the height growth response due to mycorrhizae because the initial growth effects due to the seed were probably less evident in this later growth.

The four samples of each lot were then analyzed as follows. The seedlings in each sample were counted and their green and dry weight determined. Finally each sample was analyzed[3] for total nitrogen, phosphorus, and potassium by the official

Fig. 2—Section of nursery seed bed showing spotted appearance. Dark spot in front center contains mycorrhizal seedlings. Light areas are made up of non-mycorrhizal seedlings.

[3]I am indebted to the Plant Chemistry subsection, Iowa Agricultural Experiment Station, for these analyses.

methods of the Association of Official Agricultural Chemists (1).

The results of all comparisons are shown in Tables 1 and 2. The data are clear cut and there is little overlapping in the ranges displayed by the individual measurements. In all cases, however, significance was determined by the methods outlined by Snedecor (8).

Only the following salient facts shown in Tables 1 and 2 need be pointed out: where mycorrhizae were present both green and dry plant weights were doubled over the non-mycorrhizal seedlings; the mycorrhizal plants contained twice as much nitrogen and potassium and four times as much phosphorus as the others (totals per plant); on a percentage dry weight basis, there was little difference with respect to nitrogen and potassium content but the mycorrhizal plants contained twice as much phosphorus as did the non-mycorrhizal plants; total height growth of mycorrhizal plants was 35 per cent greater, and height growth from the cotyledons to the bud 60 per cent greater than that of the non-mycorrhizal seedlings; and the average mycorrhizal plant root system possessed over six hundred absorbing short roots and mycorrhizal tips or branches, while the non-mycorrhizal plants had only slightly over three hundred.

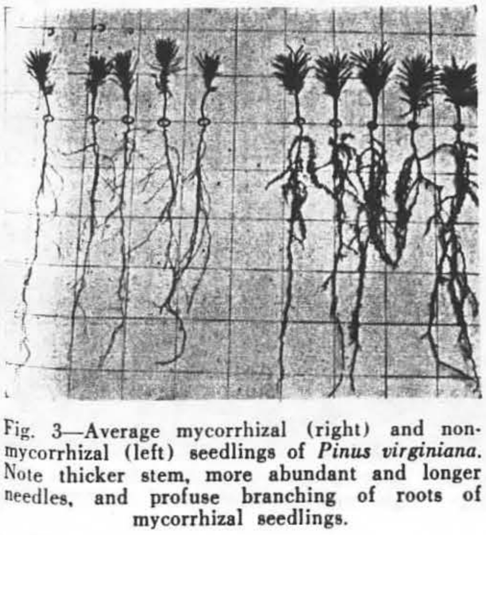

Fig. 3—Average mycorrhizal (right) and non-mycorrhizal (left) seedlings of *Pinus virginiana.* Note thicker stem, more abundant and longer needles, and profuse branching of roots of mycorrhizal seedlings.

The facts outlined indicate that the seedlings, in this soil, were unable to obtain an adequate supply of phosphorus without the aid of mycorrhizae. This evidence is supported by the knowledge that in the soil involved, phosphorus is the element generally limiting plant growth (7). The evidence supports the main thesis of the mineral nutrition theory of Stahl (9) and Hatch (3), although there is no evidence of the specific physiological mechanism or balance involved. Phosphorus was obviously limiting growth and nitrogen and potassium were apparently adequate for seedling growth at the existing level of soil phosphorus. The slight piling up of nitrogen in the non-mycorrhizal seedlings (Table 2) may have been due to the fact that phosphorus was limiting all growth and hence indirectly also limiting the use of nitrogen.

The origin and identity of the specific fungus or fungi involved in the mycorrhizal formation are not known. On the basis of the facts set forth it is reasonable to assume that this particular nursery soil did not contain fungi forming mycorrhizae on Virginia pine, nor mycorrhizae-forming fungi for the other pines planted. Hatch in another paper (2) reports the lack of mycorrhizae-forming fungi in a plains soil from the vicinity of Cheyenne, Wyoming, and reviews instances of prairie soil in Australia and Rhodesia lacking mycorrhizal fungi and supporting exceptionally poor seedling growth. It is probable that the mycorrhizae formed here developed from fungi introduced with the pine litter mulch and that the original soil either did not possess the specific fungi or that long years of cultivation in agricultural crops had eliminated them.

Regardless of the method of introduction of the fungi, mycorrhizae began to form during the growing season and their

TABLE 1

AVERAGE HEIGHT GROWTH AND MYCORRHIZAL DEVELOPMENT OF MYCORRHIZAL AND NON-MYCORRHIZAL PINUS VIRGINIANA SEEDLINGS

Sample	Number of seedlings	Height growth: Root collar to cotyledons (*inches*)	Cotyledons to bud (*inches*)	Total (*inches*)	Short roots and mycorrhizal development[3]: Number mycorrhizal short roots	Number non-mycorrhizal short roots	Total short roots
Mycorrhizal	20	1.34±.05[1]	1.36±.08	2.70±.09	350±28	321±22	672±32
Non-mycorrhizal	20	1.15±.06	0.85±.04	2.00±.06	7±2.1	297±15	304±15
Difference		0.19	0.51	0.70	343	24	368
T values[2]		2.65[4]	5.86[5]	6.3[5]	11.9[5]	0.88	10.2[5]

[1]Standard error.
[2]T values computed for 38 degrees freedom.
[3]Root classification of Hatch and Doak (4).
[4]Significant—below the 5 per cent level.
[5]Highly significant—below the 1 per cent level.

TABLE 2

WEIGHT AND NUTRIENT CONTENT OF MYCORRHIZAL AND NON-MYCORRHIZAL SEEDLINGS OF *Pinus Virginiana* GROWN IN A PRAIRIE NURSERY

Sample	Number of seedlings	Green weight of ave. plant (*mgs.*)	Dry weight of ave. plant (*mgs.*)	Nitrogen content: Per cent dry weight	Nitrogen content: Ave. per plant (*mgs.*)	Phosphorus content: Per cent dry weight	Phosphorus content: Ave. per plant (*mgs.*)	Potassium content: Per cent Dry weight	Potassium content: Ave. per plant (*mgs.*)
Mycorrhizal									
1	25	1228	322	1.79	5.76	0.178	0.57	0.64	2.06
2	25	1216	307	1.72	5.28	0.182	0.56		
3	40	1232	329	1.80	5.92	0.186	0.61	0.65	2.14
4	50	1246	333	1.81	6.03	0.192	0.64	0.69	2.30
Average		1230±6.1[1]	323±5.7	1.78±.019	5.75±.17	0.184±.003	0.60±.02	0.66±.015	2.17±.07
Non-mycorrhizal									
1	50	626	165	1.85	3.05	0.089	0.15	0.65	1.07
2	50	566	148	1.85	2.74	0.092	0.14	0.64	0.95
3	50	574	147	1.85	2.75	0.099	0.15	0.63	0.93
4	32	600	150	1.96	2.94	0.110	0.16	0.59	0.88
Average		592±13.6	152±4.2	1.88±.027	2.87±.08	0.097±.005	0.15±.004	0.63±.013	0.96±.04
Differences of averages		639.0	171		2.88		0.45		1.21
T values[2]		42.9[4]	23.9[4]	2.67[3]	15.8[4]	15.8[4]	28.1[4]	1.62	15.9[4]

[1]Standard error.
[2]T values computed for 6 degrees of freedom.
[3]Significant—below the 5 per cent level.
[4]Highly significant—below the 1 per cent level.

presence became evident as soon as the seedlings reached the point where growth depended upon normal physiological processes rather than on food materials stored in the seed. No data are available regarding the spread of mycorrhizal fungi through the soil but it is believed from observation during the latter part of the growing season that the size of the infected spots increased. All the roots possessing mycorrhizae were profusely branched, had a large number of absorbing short roots, and the mycorrhizal short roots showed multiple dichotomous branching. The net result was apparently a much larger and more efficient absorbing system than was available to the non-mycorrhizal plants.

Summary

At Ames, Iowa, a new nursery was established on an area that had previously been farmed for many years. In this nursery various species of conifers including a number of pine species were planted on a sandy loam soil having a pH of 6.0 to 6.2. Four species of pines were mulched with pine needles taken from a nearby plantation. In the middle of the first growing season many of the pine seedlings turned brown to reddish purple in color while certain spots in the beds retained their normal color. The normal seedlings continued growth but the others ceased growth almost entirely. Upon investigating the roots of the trees it was found that mycorrhizae were uniformly present on all the seedlings that were normally colored and had continued growth, while the stunted seedlings lacked mycorrhizae.

Attention was concentrated on Virginia pine (*Pinus virginiana* x Miller) in obtaining quantitative data and attempting to define the relationships involved. Samples of mycorrhizal and non-mycorrhizal seedlings were taken and analyzed for green and dry weight, height growth, and amount of nitrogen, phosphorus, and potassium present in plant tissues.

It is concluded that the differences in plant development were due to differences in the availability of phosphorus and that the mycorrhizae had been the agency which enabled the affected seedlings to absorb this element at a more rapid rate than the non-mycorrhizal plants. The data obtained support the mineral nutrition theory of mycorrhizae of Stahl (9) and Hatch (3).

No information was obtained as to the specific fungi involved in the mycorrhizae formation but it is believed that they were introduced with pine needle mulch taken from a vigorous plantation in the vicinity of the nursery.

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